THE EFFECT OF Na^+ AND K^+ ON GLYCOLYTIC ENZYMES: DIFFERENTIAL RESPONSE OF PYRUVATE KINASE FROM DOG AND HUMAN ERYTHROCYTES

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1. Introduction

Human erythrocytes contain high potassium and low sodium ion concentrations (136 and 19 meq/1, respectively), while dog erythrocytes maintain these ions in an opposite proportion [1,2]. In view of these striking differences, it is of interest to examine the role of erythrocyte ion composition in the regulation of glycolysis.

In a previous communication we have demonstrated a direct cation effect on glycolysis, utilising hemolysates which were reconstituted following gel filtration [3]. It was shown that in the human erythrocytes glycolysis is markedly stimulated by K^+, while Na^+ counteracts this effect. In contrast, the glycolytic system in the dog erythrocyte does not respond to either Na^+ or K^+. It is expected that such a species-dependent difference of the glycolytic system reflects differential sensitivities of rate limiting enzymes to specific cation composition.

The present study compares the cation sensitivity of hexokinase, phosphofructokinase and pyruvate kinase in dog and human erythrocytes. It is demonstrated that, indeed, pyruvate kinase in the two species not only shows different kinetic properties, but also displays different sensitivity to the monocations.

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2. Materials and methods

2.1. *Preparation of gel-filtered hemolysate*

Human and dog venous blood was obtained in heparin. All further manipulation were conducted at 4°C. The cells were washed three times with 155 mM NaCl solution and the top layer along with the buffy coat were discarded. The washed cells were hemolysed by diluting a volume of the packed cells with an equal volume of distilled water. The ghosts were removed by centrifugation at 27 000 *g* for 20 min to obtain the cell-free hemolysate. A column of Sephadex G-25 (Fine) measuring 30 × 1 cm was equilibrated with 50 mM Tris·HCl, pH 7.8, and 1 mM glutathione. Cell free hemolysate (10 ml) was passed through the column at a rate of 2 ml per min and following the void volume, a fraction of 12 ml was collected. This gel-filtered hemolysate contained no detectable ATP, while Na^+ and K^+ concentrations were below 2 mM.

2.2. *Activity measurement*

For the determination of glycolytic activity, hemolysates (2 ml) were incubated at 37°C for 1 hr in a final volume of 3.5 ml containing 2 mM glucose, 0.6 mM ATP, 0.6 mM ADP, 0.15 mM NAD, 0.15 mM NADH, 0.6 mM NADP, 1.2 mM $MgCl_2$ and 3 mM phosphate buffer (either Na^+ or K^+) pH 7.8. Where indicated, NaCl and/or KCl were added. Glucose was determined by the glucose-oxidase method [4]. Hexokinase activity and phosphofructokinase activity were measured as previously described [5]. Pyruvate

kinase was assayed at 25°C by a modification of the method of Bücher and Pfliederer [6]. The standard assay solution (3.0 ml) contained 8.3 mM triethanolamine HCl, pH 7.5; 8.0 mM $MgSO_4$; 0.4 mM ADP; 1000 Bücher units of lactate dehydrogenase; 0.2 mM NADH; 2.0 mM phosphoenolpyruvate (PEP) and K^+ or Na^+ as indicated. Reactions were followed spectrophotometrically at 340 nm.

Purification of human and canine red cell pyruvate kinase was performed according to Munro [7]. A 400-fold purification was achieved. Following DEAE cellulose chromatography and ammonium sulfate fractionation, the final precipitate was dissolved in a solution containing 0.125 M KCl, 0.01 M potassium-phosphate, 7 mM $MgSO_4$ and 8 mM triethanolamine buffer, pH 7.0. The enzyme was stored at −20°C and used within 5 days. Prior to use, K^+ was removed by gel filtration with a column (30 × 20 cm) of Sephadex G-25. The enzyme was eluted with a solution containing 8 mM triethanolamine buffer pH 7.0 and 7 mM $MgSO_4$.

2.3. *Materials*

All chemicals were purchased from Sigma Chemicals Co., St. Louis. Sephadex G-25 was obtained from Pharmacia Fine Chemicals, Uppsala.

3. Results

Hexokinase, phosphofructokinase and pyruvate kinase are known to be rate limiting in the glycolytic pathway or erythrocytes [8]. Therefore, the effect of KCl on the activities of these enzymes in hemolysate was explored. Table 1 shows that the addition of KCl in a concentration which increased glycolysis in human hemolysates, did not affect hexokinase and phosphofructokinase activities, but had a pronounced stimulatory effect on pyruvate kinase. In dog hemolysates, neither pyruvate kinase nor glucose consumption were affected by the addition of KCl.

It is thus evident that pyruvate kinase is intimately involved in the erythrocyte response to monocations. Therefore, some of the kinetic properties of this enzyme, purified from canine and human hemolysate, were explored with particular reference to K^+ and Na^+ concentrations. The substrate saturation curve of the human pyruvate kinase is sigmoidal, typical for many regulatory enzymes [7]. Fig.1 shows that the substrate saturation curve of the canine enzyme is hyperbolic: the double reciprocal plot of $1/v$ vs $1/s$ is linear.

The effect of cations on the enzyme activity was studied at two substrate concentrations, one close to the K_M value (0.45 mM) and the other at saturation level (2.25 mM). Fig.2 shows that at either substrate concentrations, human pyruvate kinase activity is clearly enhanced by 50 mM K^+ while Na^+ inhibits the activity of the enzyme. Furthermore, Na^+ counteracts the stimulatory effect of K^+. This cation effect is similar to the one observed on overall glycolysis [3]. The canine pyruvate kinase is, however, completely unaffected by K^+ or Na^+ at either 0.45 or 2.25 mM

Table 1
K^+ effect on the rate of glycolysis and on activities of rate limiting enzymes in dog and human hemolysates

	Activity, μmol × gHb^{-1} × hr^{-1}			
	Dog		Human	
	−KCl	+KCl	−KCl	+KCl
Hexokinase	5.1	5.1	4.5	5.1
Phosphofructokinase	177.6	189.0	246.0	256.8
Pyruvate kinase	244.2	250.8	243.9	426.0
Glucose consumption	9.6	9.3	7.5	10.7

The enzymatic activities were assayed in diluted hemolysate ([K^+] = 5 meq/l). Glycolytic rates were determined in reconstituted hemolysates following gel filtration as described in Methods. KCl concn. = 100 mM.

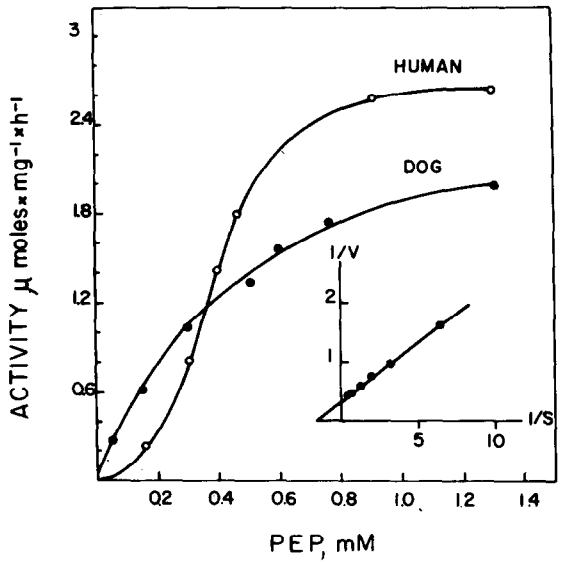

Fig.1. Substrate saturation curve of pyruvate kinase purified from human and dog erythrocytes. A double reciprocal plot for the dog system is also given.

concentration of phosphoenolpyruvate (fig.3).

Fructose-1,6-diphosphate (FDP) is known to be an allosteric activator of human hemolysate pyruvate kinase [9]. Fig.4 shows that human pyruvate kinase is stimulated by FDP, irrespective of whether K^+ is

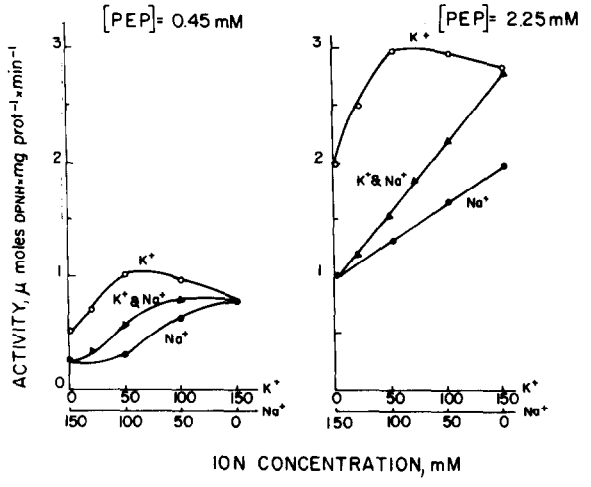

Fig.2. Activity of pyruvate kinase purified from human erythrocytes as affected by Na^+ and K^+ at two substrate concentrations.

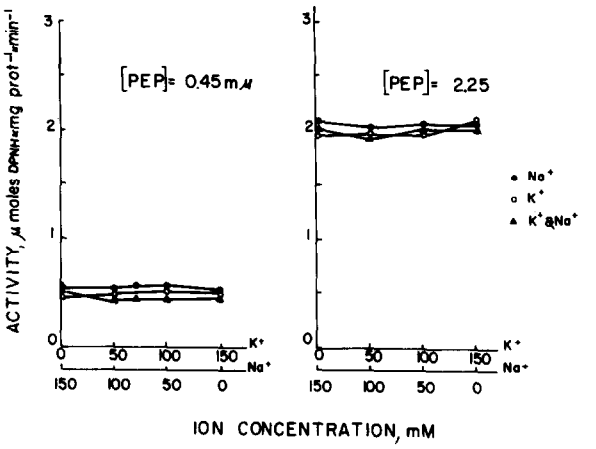

Fig.3. Activity of pyruvate kinase purified from dog erythrocytes as affected by Na^+ and K^+ at two substrate concentrations.

present (100 mM) or totally absent. These data are compatible with the presence of two independent activation sites on the enzyme, one for K^+ and one for FDP. In contrast, fig.4 demonstrated that the canine enzyme is not affected at all by FDP, regardless of K^+ concentration. Evidently, a kinetically different pyruvate kinase operates in dog as contrasted to human erythrocytes. The differences are not limited only to substrate velocity relations, but are reflected also in

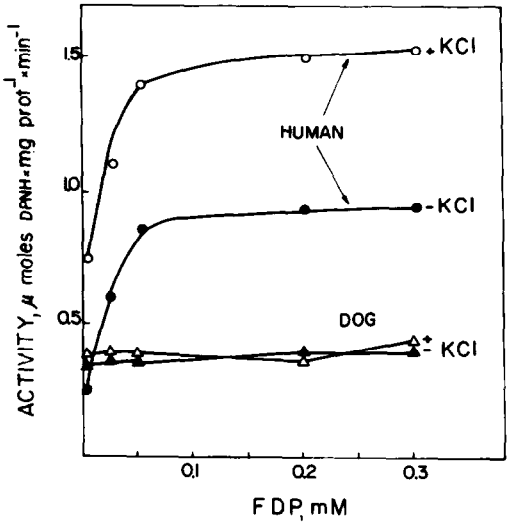

Fig.4. The effect of fructose-1,6-diphosphate on the activity of purified pyruvate kinase from human and dog red cells.

the insensitivity to the two activators, FDP and K^+. These features may, in turn, indicate the absence of regulatory sites on the canine enzyme. However, actual measurements of binding of K^+ and FDP to the enzyme are required to establish this conclusion firmly. From biological standpoint it is also of interest to establish whether the dog erythrocyte is a prototype for other carnivores which are characterized by high [Na^+] and low [K^+].

In conclusion, the dog erythrocytes represent an interesting biological system: Coupled with a cation composition similar to that in the serum, these erythrocytes maintain an ATPase [10] and a pyruvate kinase which are neither dependent nor stimulated by Na^+ and K^+.

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